

***Veloporphyrellus latisporus*, a new generic record for India**

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ABSTRACT—During routine macrofungal surveys in various temperate forests of Indian Himalayan belt, we made some collections of the interesting genus *Veloporphyrellus*. After thorough literature review, morphological examinations, and phylogenetic analysis of two ribosomal genes, those collections were found to be conspecific with *Veloporphyrellus latisporus*, which was described from coniferous forests of Pakistan. We present the genus and the species from India for the first time with detailed morphological description, illustrations, multigene phylogenetic analysis, and comparisons with related species.

KEY WORDS—*Austroboletoidae*, *Boletaceae*, Himachal Pradesh, Jammu & Kashmir, taxonomy

Introduction

Veloporphyrellus L.D. Gómez & Singer (*Boletaceae*), was typified by *V. pantoleucus* L.D. Gómez & Singer, described from Costa Rica (Gómez & Singer 1984). Subsequently, nine other *Veloporphyrellus* species were described, from Asia, Africa, and North America (<http://www.indexfungorum.org/>

names/names.asp). Combination of characters like pinkish or greyish pink hymenophore, pileus margin with a membranous veil, smooth basidiospores and trichodermous nature of pileipellis make this genus morphologically distinct within *Boletaceae*. Moreover, multigene phylogenetic analysis placed *Veloporphyrellum* species within the subfamily *Austroboletioideae* (Wu & al. 2014), although the genus is polyphyletic (Li & al. 2014, Wu & al. 2016, Gelardi & al. 2021, Ayala-Vásquez & al. 2022).

Being a megadiverse country, India exhibits a potential macrofungal diversity along with its floral and faunal diversity. Members of an ectomycorrhizal family like *Boletaceae* are explored time to time from different part of Himalayas (Berkeley 1852, Lakhanpal 1996, Sharma & al. 2005, Das 2009, 2012, 2013; Kour & al. 2013, Chakraborty & Das 2015, Das & al. 2014, Chakraborty & al. 2018). During routine macrofungal surveys to the different parts of Western Himalayan region ranging from Jammu & Kashmir (Kishtwar district; Ramban district) to Himachal Pradesh (Chamba district) authors came across a few interesting members of *Boletaceae*. After detail macro- and micromorphological characterization combined with phylogenetic studies based on nrITS and nrLSU sequences revealed our present collections to be *Veloporphyrellum latisporus*, which was originally described from Pakistan (Khan & al. 2021).

Here we report the genus *Veloporphyrellum* as well as the species *V. latisporus* for the first time from India, based on a detailed morphological description, illustration, and multi-gene phylogenetic inference.

Materials & methods

Morphological analysis

Young to mature basidiomata were collected during surveys in forested areas of Jammu & Kashmir and Kalatop Wildlife Sanctuary (Himachal Pradesh) during the monsoon season, July and August, in 2021–22. Macromorphological characterizations were noted in the field or at basecamp from fresh and dissected basidiomata. Photographs were taken in the field with a Canon Power Shot SX 50 HS camera. Color codes and terms mostly follow Kornerup & Wanscher (1978). Samples were dried in a field drier. Micromorphological characters were observed after mounting freehand sections of dried materials in a solution of 5% KOH, 1% Phloxin, and 1% ammoniacal Congo red with an Olympus CX 41 compound microscope. Drawings of the micromorphological features were made with the help of drawing tube at 1000× magnification. Microscopic photographs were taken with an Olympus BX 53 camera. The basidiospores were measured in lateral view. Basidiospore measurements and length/width ratios (Q) are recorded as: minimum–mean–maximum. Basidium length excludes the length of sterigmata. The specimens are conserved in the Central National Herbarium, Botanical Survey of India, Howrah, India (CAL) and the

Herbarium, Centre of Biodiversity and Taxonomy, University of Kashmir, Srinagar, India (KASH).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from 100 mg of dried basidiomata with the InstaGene™ Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. The nrITS and nrLSU genes regions were amplified with primer pairs ITS-1F and ITS-4 (White & al. 1990; Gardes & Bruns 1993) and LR0R and LR5 (Vilgalys & Hester 1990), respectively. PCR amplification was performed on a thermal cycler (Eppendorf, Germany) programmed for 5 min at 95°C, 30 cycles of 1 min at 95°C, 30 s at 52°C, 2 min at 72°C, and a final 7 min extension step at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers. The sequence quality was checked using Sequence Scanner Software v. 1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious v. 5.1 (Drummond & al. 2010). The newly generated sequences in this study were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>).

Sequence alignment and phylogenetic analysis

The newly generated nrITS and nrLSU sequences of *V. latisporus* from India and its close relatives were retrieved from the nBLAST search against GenBank (<https://www.ncbi.nlm.nih.gov/genbank>), and relevant published phylogenies (Li & al. 2014, Khan & al. 2021). Two datasets (ITS and LSU) were created separately. Both the datasets were aligned separately using the online version of MAFFT v. 7 with the L-INS-i strategy (Kato & al. 2019) and default settings. To eliminate ambiguously aligned positions in the alignment as objectively as possible, Gblocks 0.91b (Talavera & Castresana 2007) was used. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Each nrLSU and nrITS dataset were then phylogenetically analysed using the maximum-likelihood (ML) method. For the ML analysis, the concatenated alignment was carried out using raxmlGUI v. 2.0 (Edler & al. 2021) with the GTRGAMMA substitution model. The ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. Maximum-likelihood bootstrap values $\geq 70\%$ are shown in both phylogenetic trees. *Tylopilus fellus* was used as the outgroup taxon for both analyses.

Phylogeny

The ITS data matrix contained 16 sequences and the alignment comprised 962 characters. The LSU matrix contained 27 sequences and the alignment comprised 1357 characters.

In our nrITS-based phylogenetic estimation (FIG. 1), sequences derived from Indian collections nested within the clade of five Pakistani *Veloporphyrillus latisporus* collections. In our nrLSU-based phylogenetic estimation (FIG. 2),

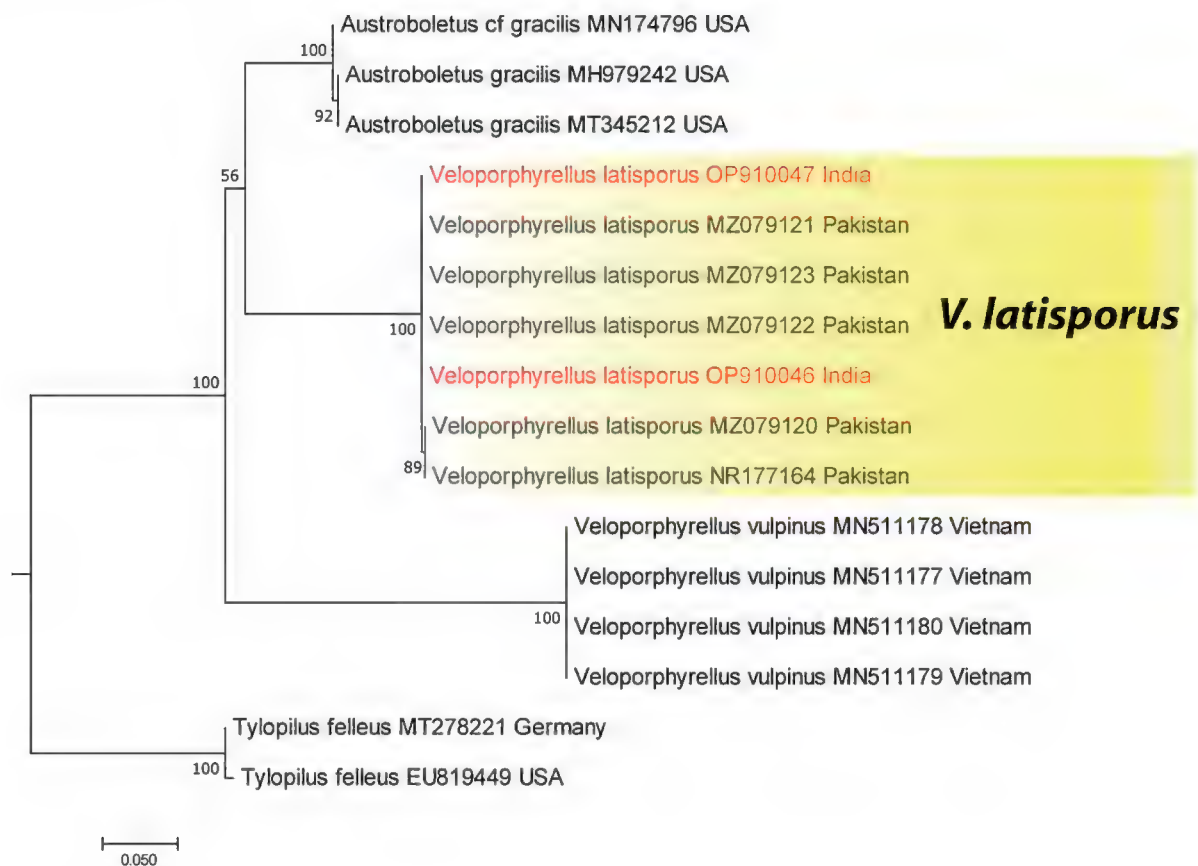


FIG 1: Maximum likelihood phylogenetic tree generated from the analysis of the nrITS sequences of *Veloporphyrrellus latisporus* and closely allied species. Maximum likelihood bootstrap support values $\geq 70\%$ are shown at branch-nodes.

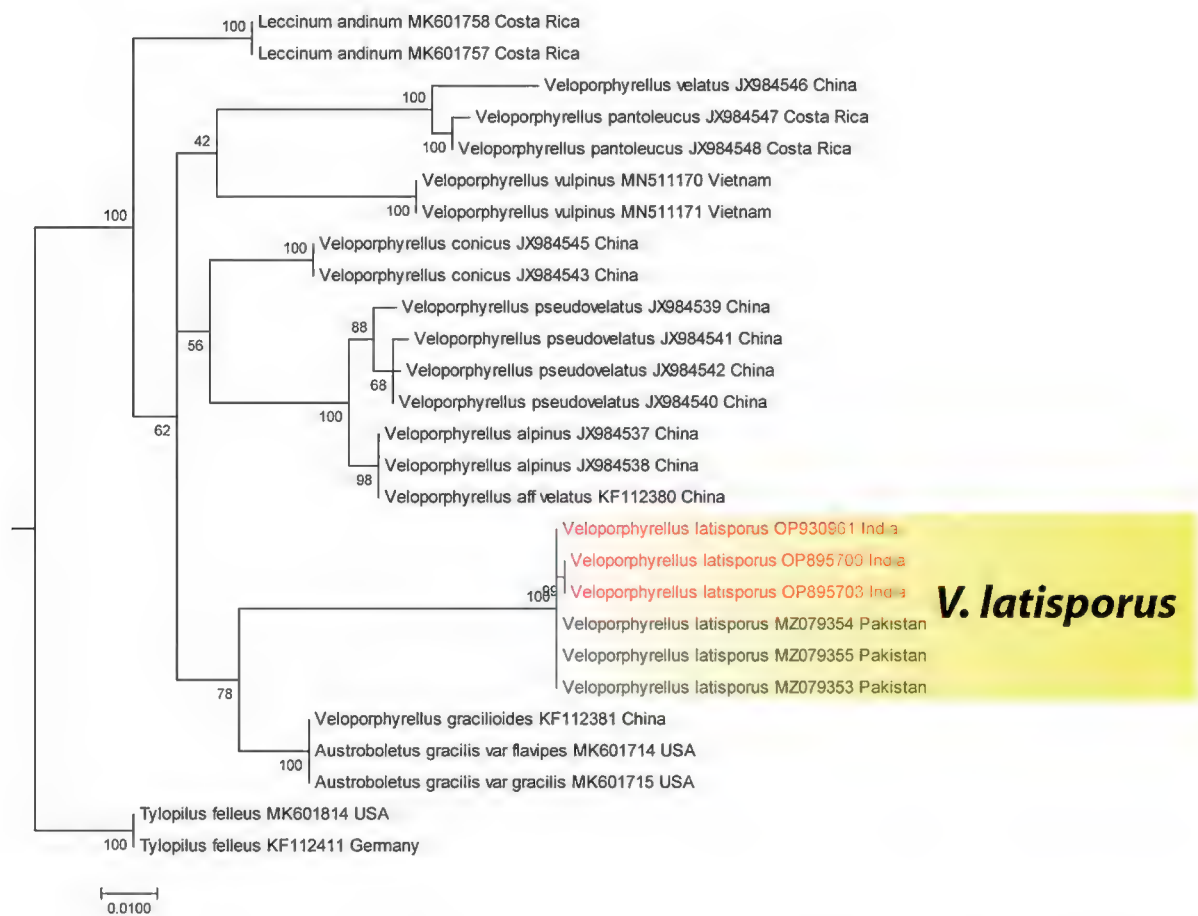


FIG 2: Maximum likelihood phylogenetic tree generated from the analysis of the nrLSU sequences of *Veloporphyrrellus latisporus* and closely allied species. Maximum likelihood bootstrap support values $\geq 70\%$ are shown at branch-nodes.

Veloporphyrellum is better resolved; and sequences derived from our Indian collections nested within the clade consisting of three Pakistani *V. latisporus* collections. Both phylogenetic analyses strongly suggest that our Indian collections are conspecific with the South Asian *V. latisporus*.

Taxonomy

Veloporphyrellum latisporus J. Khan & S. Ullah, *Nordic J. Bot.* 39(9): e03178.
2021.

FIGS 3, 4



FIG 3: *Veloporphyrellum latisporus* (KASH 8619 [A]; CAL 1905 [B–G]). A–C. Basidiomata in field and in basecamp; D. Pileipellis; E. Basidia; F. Cheilocystidia; G. Basidiospores. Scale bars: D = 50 µm; E–G = 10 µm.

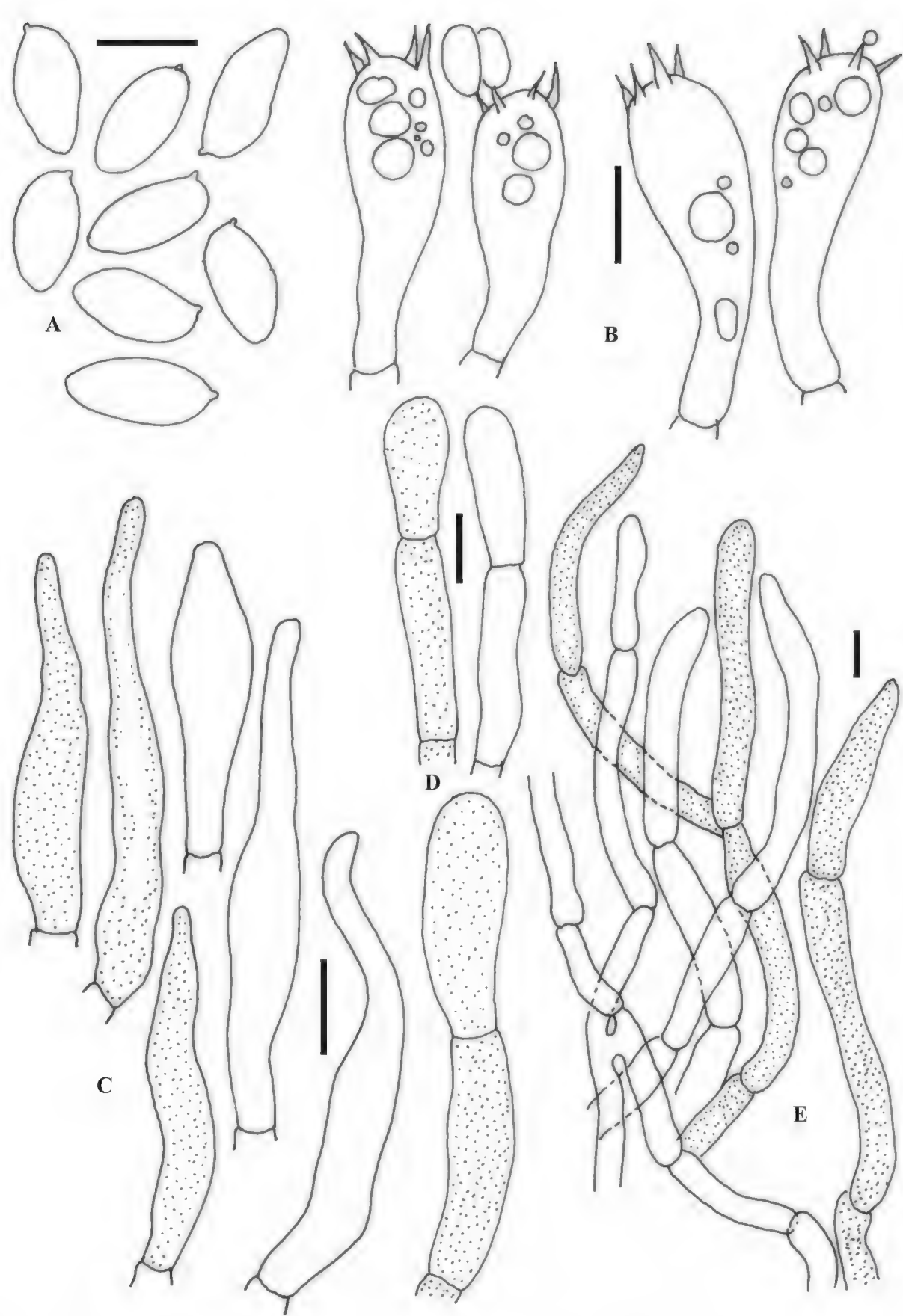


FIG 4: *Veloporphyrellum latisporus* (CAL 1905). A. Basidiospores; B. Basidia; C. Pleurocystidia; E. Cheilocystidia; F. Pileipellis. Scale bars= 10 μ m.

BASIDIOMATA epigeous, medium-sized. PILEUS 50–78 mm broad, convex, plano-convex with age; margin plane, velar remnants present as a sterile flap of tissues, entire, sometimes cracked; surface dry, smooth at first, then becoming rough to areolate and sub-tomentose to squamulose, cracked with maturity, reddish brown (8D–E4–7) to brownish red (10D4–7). PILEUS CONTEXT up to 47 mm thick at the disc, thinning towards the margin, pale yellow (3A2), unchanging when cut or bruised. TUBE up to 3 mm deep, adnate, pinkish white; pore angular, 1–3/mm. STIPE 60–90 × 8–10 mm, central, straight to curved, cylindrical, broader at base; surface dry, non-viscid, very finely striate, brownish red to reddish brown (8E7–8). STIPE CONTEXT solid, white, unchanging when cut bruised. BASAL MYCELIUM white. TASTE None.

BASIDIOSPORES 12–12.5–14 × 5.5–6–6.7 µm, ($n = 40$; $Q = 1.97–2–2.22$), boletoid, appearing smooth under light microscope, pale olivaceous when observed in KOH and H₂O. BASIDIA 28–38 × 10–11 µm, clavate to subclavate, thin-walled, 4-spored. PLEUROCYSTIDIA 38–63 × 6–10 µm, subclavate to narrowly clavate, fusiform to narrowly ventricose, thin-walled, mostly colorless in KOH and H₂O, few are pigmented. LAMELLAR EDGE fertile with basidia and cheilocystidia. CHEILOCYSTIDIA 46–84 × 8–13 µm, subclavate to subcylindrical, septate, abundant. HYMENOPHORAL TRAMA composed of colorless and hyaline cylindrical hyphae. PILEIPELLIS trichodermous, composed of erect or ascending, interwoven, septate hyphal terminations; terminal elements measuring 33–54 × 7–9 µm, mainly cylindrical or sometimes narrow at the apex, apically rounded, thin walled. CAULOCYSTIDIA not found. CLAMP CONNECTIONS absent in all tissues.

ECOLOGY & DISTRIBUTION—occurrence under moist coniferous forests; known from Pakistan and India.

SPECIMENS EXAMINED—INDIA. HIMACHAL PRADESH: **Chamba district**, Kalatop Wildlife sanctuary, alt. 2398 m, on soil under *Pinus wallichiana* A.B. Jacks., 28 Aug. 2021, D. Chakraborty DC 21-75 (CAL 1905; GenBank OP910046, OP895703); JAMMU & KASHMIR: **Kistwar district**, Bindraban, alt. 1410 m, on soil under *Pinus wallichiana* A.B. Jacks., 10 July. 2022, F. Mushtaq FM 22-0010 (CAL 1906; GenBank OP910047, OP895709); **Ramban district**, Gool, alt. 2200 m, on soil under *Abies pindrow* (Royle ex D. Don) Royle, 31 August 2022, W.S. Malik 41-Aug-22 (KASH 8619; GenBank OP930961).

Discussion

A combination of macro- and micromorphological characters identify our Indian collections as *Veloporphyrillus latisporus*: small to medium-sized basidiomata with areolate to granulose, reddish brown to brownish red pileus; pinkish buff hymenophore; unchanging context color; white basal mycelium;

trichodermous nature of pileipellis; occurrence under conifers like *Pinus* and *Abies*; and multigene (nrITS and nrLSU) phylogenetic analyses undoubtedly identify the present species as *V. latisporus*. In the ITS analysis, the *V. latisporus* clade is sister to a clade consisting of *Austroboletus gracilis* and *A. cf. gracilis* (FIG. 1). In the LSU analysis, the *V. latisporus* clade is sister to a clade consisting of *V. gracilioides*, *A. gracilis* var. *gracilis* and *A. gracilis* var. *flavipes* (FIG. 2). Morphologically, our Indian specimens are mostly in conformity with the *V. latisporus* holotype described from our neighboring country Pakistan (Khan & al. 2021). However, the velar remnants of the Indian specimens are present at the pileus margin as a thin sterile flap of tissue, whereas they are reported as “not observed” in the Pakistani collections.

Morphologically, *Veloporphyrillus latisporus* can be confused in the field with some other Asian species such as *V. alpinus* Yan C. Li & Zhu L. Yang, *V. pseudovelatus* Yan C. Li & Zhu L. Yang, or *V. vulpinus* T.H.G. Pham & al., but *V. alpinus* can be separated by its distribution pattern (e.g., its occurrence in higher elevations, 3100–3600 m asl) and its sharply umbonate pileus (Li & al. 2014). *Veloporphyrillus pseudovelatus* differs from our species of interest by its sub-conical, chocolate-brown pileus and prominent velar remnants at pileus margin (Li & al. 2014); and *V. vulpinus* is phylogenetically well separated from *V. latisporus* and can also be distinguished by its bitter taste (Pham & al. 2019).

Acknowledgements

The authors are grateful to the Director of the Botanical Survey of India, Kolkata for providing facilities. One of the authors (DC) is thankful to the Science and Engineering Research Board (SERB) for providing the National Post-Doctoral Research Fellowship (File no.: PDF/2019/000917). Another author (WSM) is thankful to CSIR, New Delhi for providing Senior Research Fellowship. Dr. A. Ghosh and Mrs. B. Chakraborty are also acknowledged for their help in field collection and in preparation of the manuscript.

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